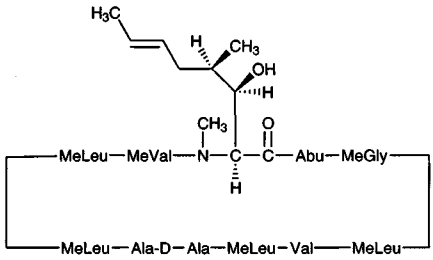
# Cyclosporine

**Molecular formula:** $C_{62}H_{111}N_{11}O_{12}$
**Molecular weight:** 1202.64
**CAS Registry No.:** 59865-13-3
**Merck Index:** 2821




## SAMPLE

**Matrix:** blood
**Sample preparation:** Mix 1 ml whole blood with 100 µL 1 µg/mL cyclosporin D in EtOH, 2 mL 180 mM HCl and 7 mL diethyl ether. Shake for 5 min and centrifuge at 4200 g for 5 min. Remove the ether phase and add it to 2.5 mL 100 mM NaOH, shake and centrifuge briefly. Evaporate the ether phase to dryness under a stream of nitrogen. Reconstitute the residue with 200 µL mobile phase, inject a 200 µL aliquot.

## HPLC VARIABLES

**Column:** 250 × 4.6 5 µm Spherisorb S5 CN normal phase
**Mobile phase:** Hexane:isopropanol 90:10
**Column temperature:** 50
**Flow rate:** 1.45
**Injection volume:** 200
**Detector:** UV 212

## CHROMATOGRAM

**Retention time:** 8.9
**Internal standard:** cyclosporin D (7.1)
**Limit of detection:** 10 ng/mL
**Limit of quantitation:** 20 ng/mL

## OTHER SUBSTANCES

**Extracted:** metabolites

## KEY WORDS

whole blood; normal phase

## REFERENCE

Khoschsorur,G.; Semmelrock,H.J.; Rödl,S.; Auer,T.; Petek,W.; Iberer,F.; Tscheliessnigg,K.H. Rapid, sensitive high-performance liquid chromatographic method for the determination of cyclosporin A and its metabolites M1, M17 and M21, *J.Chromatogr.B*, **1997**, *690*, 367–372.

## SAMPLE

**Matrix:** microsomal incubations
**Sample preparation:** 500 µL Microsomal incubation + 500 µL MeCN, mix, centrifuge, inject a 100 µL aliquot of the supernatant.

## HPLC VARIABLES

**Guard column:** ultra sepharose ODS (Beckman)
**Column:** 250 × 4.6 ultra sepharose ODS (Beckman)
**Mobile phase:** Gradient. MeCN:water from 55:45 to 60:40 over 15 min, to 70:30 over 10 min, to 90:10 over 15 min, return to initial conditions over 5 min.
**Column temperature:** 70
**Injection volume:** 100

**CHROMATOGRAM**
**Retention time:** 34.4 (cyclosporin G)

**OTHER SUBSTANCES**
**Extracted:** metabolites

**KEY WORDS**
human; liver

**REFERENCE**
Pichard,L.; Domergue,J.; Fourtanier,G.; Koch,P.; Schran,H.F.; Maurel,P. Metabolism of the new immunosuppressor cyclosporin G by human liver cytochromes P450, *Biochem.Pharmacol.*, **1996**, *51*, 591–598.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Prepare a solution in MeCN:water 50:50, inject a 20 μL aliquot.

**HPLC VARIABLES**
**Column:** 250 × 4.6 3 μL Hypersil ODS
**Mobile phase:** MeCN:water:MTBE 34:59:7.2 containing 20 mM phosphoric acid and 10 mM sodium dodecyl sulfate, adjusted to pH 2.8 with concentrated NaOH solution (A) or MeCN:water:MTBE 43:52:5 containing 20 mM phosphoric acid and 10 mM dodecyl sulfate, adjusted to pH 5.0 with concentrated NaOH solution (B)
**Column temperature:** 80
**Flow rate:** 2 (A), 1.7 (B)
**Injection volume:** 20
**Detector:** UV 210

**CHROMATOGRAM**
**Retention time:** 28 (A), 29.5 (B) (cyclosporin A)

**OTHER SUBSTANCES**
**Simultaneous:** cyclosporin B, cyclosporin C, cyclosporin D, cyclosporin F, cyclosporin G, cyclosporin H, cyclosporin L, cyclosporin U, isocyclosporin A, Leu$^4$-cyclosporin, dihydroMeBmt$^1$-cyclosporin

**REFERENCE**
Husek,A. High-performance liquid chromatographic analysis of cyclosporin A and its oral solution, *J.Chromatogr.A*, **1997**, *759*, 217–224.

**SAMPLE**
**Matrix:** solutions

**HPLC VARIABLES**
**Column:** 250 × 4.6 10 μm Spherisorb ODS-2
**Mobile phase:** MeCN:water 70:30
**Flow rate:** 2
**Detector:** UV 215

**CHROMATOGRAM**
**Retention time:** 10

**REFERENCE**
Mithani,S.D.; Bakatselou,V.; TenHoor,C.N.; Dressman,J.B. Estimation of the increase in solubility of drugs as a function of bile salt concentration, *Pharm.Res.*, **1996**, *13*, 163–167.

## SAMPLE

**Matrix:** tissue

**Sample preparation:** Freeze four 50 μL portions of scales with liquid nitrogen and grind with purified quartz sand. Vortex the mixture in two portions. Vortex each portion twice with 2 mL MeCN for 3 min, centrifuge at 3500 rpm for 5 min. Filter (0.2 μm) the supernatant, evaporate it to dryness under the stream of nitrogen. Reconstitute the residue with 5 mL MTBE. Vortex with 2 mL 100 mM NaOH for 3 min, filter (0.2 μm), and wash the organic phase with 2 mL 100 mM HCl. Evaporate the MTBE under a stream of nitrogen. Dissolve the residue in 300 μL MeCN, dilute with 900 μL water and 200 μL mobile phase A. Filter, wash filter with 500 μL mobile phase A. Inject a 1.9 mL aliquot onto column A, elute with mobile phase A. Divert the effluent having retention time 16-25 min onto column B. Wash column B with 1 mL water, dry with a stream of pure nitrogen. Elute with 1 mL MeCN in the opposite direction. Evaporate the clear eluate to dryness, reconstitute the residue in 100 μL MeCN, add 100 μL water and IS. Inject a 20 μL aliquot onto column C and elute with mobile phase B or C.

## HPLC VARIABLES

**Column:** A 20 × 4 5 :gmm Spherisorb Alumina PC18 purification cartridge (Bischoff Analysentechnik, Germany); B PRP-1 trap cartridge (Bischoff Analysentechnik, Germany); C 4 × 4 5 μm LiChrospher 100 RP-18 + 250 × 4.6 5 μm LiChrospher 100 RP-18

**Mobile phase:** A MeCN:water 29:71 containing 200 mM NaOH, pH 11; B MeCN:water 71:29; C Gradient. MeCN:water from 75:25 to 85:15 over 10 min

**Column temperature:** 70

**Flow rate:** 2 (A), 1 (B, C)

**Injection volume:** 20

**Detector:** UV 212

## CHROMATOGRAM

**Retention time:** 11.5 (B), 7.87 (C)

**Internal standard:** cyclosporine C (6.25 (C), 8.6 (B))

**Limit of detection:** 7 ng

## KEY WORDS

cutaneous scale

## REFERENCE

Spöttl,T.; Eibler,E.; Wiegrebe,W. ng-Determination of cyclosporine A in cutaneous scales, *Pharmazie*, **1997**, *52*, 759–762.

## SAMPLE

**Matrix:** whole blood

**Sample preparation:** Mix 2 mL whole blood with 16 μL 100 μg/mL IS in MeOH and 100 mg NaF. Vortex for 15 s, add 5 mL diethyl ether, mix for 2 min. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of air at 50°. Reconstitute the residue with 150 μL mobile phase and 400 μL hexane, vortex, inject a 20 μL aliquot.

## HPLC VARIABLES

**Column:** 200 × 4.6 7 μm Spherisorb C8

**Mobile phase:** MeCN:MeOH:water:isopropanol 57:18:25:1.5

**Column temperature:** 65

**Flow rate:** 1.4

**Injection volume:** 20

**Detector:** UV 208

## CHROMATOGRAM

**Retention time:** 4.6

**Internal standard:** cyclosporine D (5.5)

**Limit of detection:** 20 ng/mL

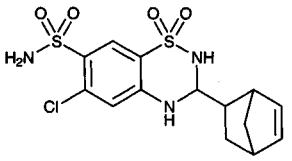
## KEY WORDS

pharmacokinetics

## REFERENCE

Li,K.; Wang,P.; Yuan,Y.; Liu,X. Determination of cyclosporin-A in human whole blood by reversed phase liquid chromatography with single-step extraction, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2179–2188.

# Cyclothiazide



**Molecular formula:** $C_{14}H_{16}ClN_3O_4S_2$
**Molecular weight:** 389.88
**CAS Registry No.:** 2259-96-3
**Merck Index:** 2822
**Lednicer No.:** 1 358

## SAMPLE

**Matrix:** solutions
**Sample preparation:** Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

## HPLC VARIABLES

**Column:** 300 × 3.9 10 μm μBondapak C18
**Mobile phase:** MeOH:acetic acid:triethylamine:water 40:1.5:0.5:58
**Flow rate:** 1.5
**Injection volume:** 10
**Detector:** UV

## CHROMATOGRAM

**Retention time:** k' 2.51

## REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

## SAMPLE

**Matrix:** solutions

## HPLC VARIABLES

**Column:** 250 × 4.6 Zorbax RX
**Mobile phase:** Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
**Column temperature:** 30
**Flow rate:** 2
**Detector:** UV 210

## OTHER SUBSTANCES

**Also analyzed:** acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine,

estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

## REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

## SAMPLE

**Matrix:** urine

**Sample preparation:** 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 μL 50 μg/mL β-hydroxyethyltheophylline in MeOH, inject 5 μL aliquot. (Solid buffer I was $KH_2PO_4$:$Na_2HPO_4$ 99:1, solid buffer II was $NaHCO_3$:$K_2CO_3$ 3:2.)

## HPLC VARIABLES

**Column:** 250 × 4.6 5 μm HP Hypersil ODS (A) or HP LiChrosorb RP-18 (B)

**Mobile phase:** Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM $NaH_2PO_4$ containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)

**Flow rate:** 1

**Injection volume:** 5

**Detector:** UV 230, UV 275

### CHROMATOGRAM

**Retention time:** 13.85 (A), 14.8 (B)
**Internal standard:** β-hydroxyethyltheophylline (3.7 (A), 4.4 (B))
**Limit of detection:** 1000 ng/mL

### OTHER SUBSTANCES

**Extracted:** furosemide, metolazone, amiloride, acetazolamide, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, hydroflumethiazide, chlorthalidone, dichlorphenamide, trichloromethiazide, methyclothiazide, benzthiazide, polythiazide, bendroflumethiazide, ethacrynic acid, bumetanide, probenecid, spironolactone, canrenone, flumethiazide
**Noninterfering:** acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

### REFERENCE

Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

### SAMPLE

**Matrix:** urine
**Sample preparation:** 5 mL Urine + 50 μL 100 μg/mL 7-propyltheophylline in MeOH + 200 μL ammonium chloride buffer + 2 g NaCl, extract with 6 mL ethyl acetate by rocking at 40 movements/min for 20 min and centrifuging at 800 g for 5 min, repeat extraction, combine organic layers, evaporate to dryness at 40° under a stream of nitrogen. Reconstitute in 200 μL MeCN:water 15:85 and inject 20 μL aliquots. (Ammonium chloride buffer was 28 g ammonium chloride in 100 mL water with the pH adjusted to 9.5 with concentrated ammonia solution.)

### HPLC VARIABLES

**Column:** 75 × 4.6 3 μm Ultrasphere ODS
**Mobile phase:** Gradient. MeCN:100 mM ammonium acetate adjusted to pH 3 with concentrated phosphoric acid. From 10:90 to 15:85 over 2 min to 55:45 over 3 min to 60:40 over 3 min. Kept at 60:40 for 1 min, decreased to 10:90 over 1 min and equilibrated at 10:90 for 2 min.
**Flow rate:** 1
**Injection volume:** 20
**Detector:** UV 270

### CHROMATOGRAM

**Retention time:** 6.6, 6.7 (two peaks)
**Internal standard:** 7-propyltheophylline (4.5)

### OTHER SUBSTANCES

**Simultaneous:** xipamide, bumetanide, acetazolamide, amiloride, bendroflumethiazide, buthiazide, benzthiazide, canrenone, caffeine, clopamide, chlorthalidone, diclofenamide, ethacrynic acid, furosemide, hydrochlorothiazide, mesocarb, morazone, polythiazide, probenecid, spironolactone, torsemide, triamterene
**Interfering:** piretanide

### REFERENCE

Ventura,R.; Nadal,T.; Alcalde,P.; Pascual,J.A.; Segura,J. Fast screening method for diuretics, probenecid and other compounds of doping interest, *J.Chromatogr.A*, **1993**, *655*, 233–242.

### SAMPLE

**Matrix:** urine

**Sample preparation:** Direct injection into column A with mobile phase A for 1 min then back flush onto column B with mobile phase B.

## HPLC VARIABLES

**Column:** A 20 × 2.1 30 μm Hypersil ODS-C18; B 250 × 4 Hypersil ODS-C18
**Mobile phase:** A Water; B Gradient. MeCN:buffer 15:85 for 1.5 min then to 80:20 over 8 min. Keep at 80:20 for 2.5 min then re-equilibrate with 15:85. (Buffer was 50 mM $NaH_2PO_4$ + 1.4 mL propylamine hydrochloride per liter adjusted to pH 3 with concentrated phosphoric acid.)
**Flow rate:** 1
**Injection volume:** 50
**Detector:** UV 230

## CHROMATOGRAM

**Retention time:** 9.6
**Limit of detection:** 20 ng/mL.

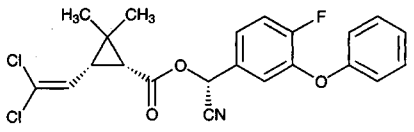
## OTHER SUBSTANCES

**Simultaneous:** bumetanide, ethacrynic acid, acetazolamide, amiloride, bendroflumethiazide, chlorthalidone, furosemide, hydrochlorothiazide, probenecid, spironolactone, triamterene

## REFERENCE

Campíns-Falco,P.; Herráez-Hernández,R.; Sevillano-Cabeza,A. Column-switching techniques for screening of diuretics and probenecid in urine samples, *Anal.Chem.*, **1994**, *66*, 244–248.

# Cyfluthrin



**Molecular formula:** $C_{22}H_{18}Cl_2FNO_3$
**Molecular weight:** 434.29
**CAS Registry No.:** 68359-37-5
**Merck Index:** 2826

## SAMPLE

**Matrix:** formulations
**Sample preparation:** Weigh out sample containing 320 mg cyfluthrin, add 25 mL dioxane (Caution! Dioxane is a carcinogen!), add 10 mL 0.65% acetophenone in hexane, shake mechanically for 30 min, add 25 mL hexane, mix well. Dilute an aliquot 1:10 with hexane, mix well, filter (0.45 μm), inject a 25 μL aliquot of the filtrate.

## HPLC VARIABLES

**Column:** 250 × 4.6 5 μm Zorbax Sil silica
**Mobile phase:** Hexane:dioxane 97.5:2.5
**Flow rate:** 1.5
**Injection volume:** 25
**Detector:** UV 230

## CHROMATOGRAM

**Retention time:** 10.7, 11.9, 12.6, 13.7 (diastereomers)
**Internal standard:** acetophenone (6.7)

## KEY WORDS

normal phase

## REFERENCE

Slahck,S.C. Liquid chromatographic method for determination of cyfluthrin in technical and formulated products, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 595–598.

## SAMPLE

**Matrix:** formulations
**Sample preparation:** Weigh out amount of formulation containing 50 mg cyfluthrin, add 20 mL 0.2% dodecaphenone in MeCN, mix by hand (emulsifiable concentrate or flowable liquid formulation) or shake mechanically for 15 min (wettable powder), filter (0,45 μm), dilute the filtrate with MeCN or mobile phase, inject an aliquot.

## HPLC VARIABLES

**Column:** 250 × 4.6 5 μm cyanopropylsilane-modified silica gel
**Mobile phase:** MeCN:water 55:45
**Flow rate:** 1.5
**Injection volume:** 10
**Detector:** UV 230

## CHROMATOGRAM

**Retention time:** 10-13
**Internal standard:** dodecaphenone (7-9)

## KEY WORDS

emulsifiable concentrate; flowable liquid; wettable powder

## REFERENCE

Harbin,D.N. Quantitation of cyfluthrin in liquid and solid formulations by reversed-phase liquid chromatography: Collaborative study, *J.AOAC Int.*, **1995**, *78*, 1335–1338.

**SAMPLE**
**Matrix:** solutions

**HPLC VARIABLES**
**Guard column:** 50 × 4 40 μm pellicular material
**Column:** 250 × 4.6 5 μm silica (IBM)
**Mobile phase:** Hexane:dichloromethane:isopropanol 99:1:0.07
**Flow rate:** 1
**Injection volume:** 10
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** k' 8.68 (trans, S), k' 9.55 (cis, R), k' 11.6 (trans, S), k' 12.9 (cis, R)

**OTHER SUBSTANCES**
**Also analyzed:** allethrin, chrysanthemol, dimethrin, ethyl chrysanthemate, permethrin, phenothrin, resmethrin, RU-11679, tetramethrin

**KEY WORDS**
normal phase

**REFERENCE**
Abidi,S.L. Column selectivity in high-performance liquid chromatography of substituted *gem*-dimethylcyclopropanes, *J.Chromatogr.*, **1986**, *368*, 59–76.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Inject an aliquot of a 0.1-1 mg/mL solution in hexane.

**HPLC VARIABLES**
**Guard column:** 5 μm Spherisorb NH2
**Column:** 250 × 4.6 Pirkle covalently-bonded column (Technicol)
**Mobile phase:** Hexane:isopropanol 99.95:0.05
**Flow rate:** 1
**Detector:** UV 230

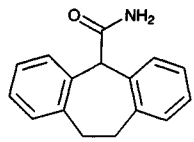
**CHROMATOGRAM**
**Retention time:** 49.0, 51.6, 59, 61.2, 64.1, 67.1, 76.0, 79.6 (enantiomers)

**OTHER SUBSTANCES**
**Also analyzed:** flucythrinate, flumethrin

**KEY WORDS**
chiral

**REFERENCE**
Lisseter,S.G.; Hambling,S.G. Chiral high-performance liquid chromatography of synthetic pyrethroid insecticides, *J.Chromatogr.*, **1991**, *539*, 207–210.

# Cyheptamide



**Molecular formula:** $C_{16}H_{15}NO$
**Molecular weight:** 237.30
**CAS Registry No.:** 7199-29-3
**Merck Index:** 2828
**Lednicer No.:** 2 222

## SAMPLE

**Matrix:** blood

**Sample preparation:** 200 μL Serum + 200 μL 25% saturated ammonium acetate, mix. Add the sample to the reservoir of a primed 4 mm/1 mL Empore C8 SPE disk cartridge suspended in a test tube (16 × 100 mm). Force the liquid then 500 μL water through the disk by centrifuging at 100-120 g for 5 min. Suspend disk cartridge in a tube, elute the drug with 100 μL MeCN and 300 μL water. Combine the eluates, inject a 50 μL aliquot.

## HPLC VARIABLES

**Guard column:** 20 × 2 30 μm Permaphase ETH (DuPont)
**Column:** 250 × 4.6 Zorbax Stable-Bond CN
**Mobile phase:** MeCN:MeOH:acetic acid:triethylamine: water 15:12.5:0.1:0.06:72.5 (Connect a 250 × 4.6 column dry packed with 37-53 μm silica gel (Whatman) as a mobile-phase saturating column between the pump and the injector .)
**Column temperature:** 50
**Flow rate:** 1.2
**Injection volume:** 50
**Detector:** UV 214

## CHROMATOGRAM

**Retention time:** 14
**Internal standard:** cyheptamide

## OTHER SUBSTANCES

**Extracted:** carbamazepine, carbamazepine diol, carbamazepine epoxide, 5-(p-hydroxyphenyl)-5-phenylhydantoin, lamotrigine, phenytoin
**Simultaneous:** acetaminophen, N-acetylprocainamide, amikacin, caffeine, chlordiazepoxide, clonazepam, desmethylchlordiazepoxide, desmethyldiazepam, diazepam, digoxin, disopyramide, erythromycin, ethosuximide, felbamate, flurazepam, gabapentin, gentamicin, lidocaine, methotrexate, nitrazepam, oxazepam, phenylethylmalonamide, phenobarbital, primidone, quinidine, salicylate, temazepam, theophylline, tobramycin, valproic acid, vancomycin

## KEY WORDS

serum; SPE; cyheptamide is IS

## REFERENCE

Lensmeyer,G.L.; Gidal,B.E.; Wiebe,D.A. Optimized high-performance liquid chromatographic method for determination of lamotrigine in serum with concomitant determination of phenytoin, carbamazepine, and carbamazepine epoxide, *Ther.Drug Monit.*, **1997**, *19*, 292–300.

## SAMPLE

**Matrix:** solutions

## HPLC VARIABLES

**Column:** 250 × 4.6 Zorbax RX

**Mobile phase:** Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
**Column temperature:** 30
**Flow rate:** 2
**Detector:** UV 210

## OTHER SUBSTANCES

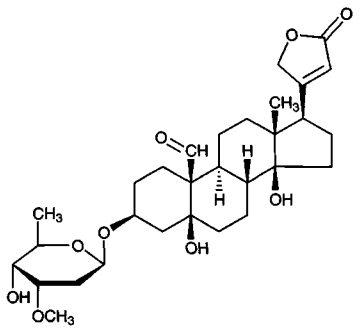
**Also analyzed:** acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

## REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

# Cymarin

**Molecular formula:** $C_{30}H_{44}O_9$
**Molecular weight:** 548.67
**CAS Registry No.:** 508-77-0
**Merck Index:** 2830



## SAMPLE

**Matrix:** solutions

## HPLC VARIABLES

**Column:** 250 × 4.6 Zorbax RX
**Mobile phase:** Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
**Column temperature:** 30
**Flow rate:** 2
**Detector:** UV 210

## OTHER SUBSTANCES

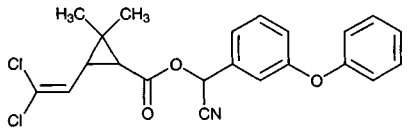
**Also analyzed:** acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazo-

cine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

## REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

# Cypermethrin



**Molecular formula:** $C_{22}H_{19}Cl_2NO_3$
**Molecular weight:** 416.30
**CAS Registry No.:** 52315-07-8
**Merck Index:** 2836

## SAMPLE

**Matrix:** bulk
**Sample preparation:** Dissolve the compound in benzene. Inject an aliquot. (Protect the sample from light! Caution! Benzene is a carcinogen!)

## HPLC VARIABLES

**Column:** 250 × 4 10 μm Lichrospher Si60
**Mobile phase:** Hexane:benzene 50:50
**Flow rate:** 1
**Detector:** UV 280; Polarimeter, diode-laser polarimetric Chiral Monitor 2000 (Applied Chromatography System, Great Britain) collimated laser diode 30 mW at 830 nm, flow cell 4.8 cm, volume 73 μL

## CHROMATOGRAM

**Retention time:** 4.92 (cis), 5.62 (trans) (UV detection); 3.06 (1RS, 3SR, SαCN diastereomer), 5.41 (1RS, 3SR, RαCN diastereomer), 5.74 (1RS, 3RS, SαCN), 6.10 (1RS, 3RS, RαCN) polarimetric detection)
**Limit of detection:** 112 μg

## OTHER SUBSTANCES

**Simultaneous:** deltamethrin, permethrin

## KEY WORDS

chiral; normal phase

## REFERENCE

Díaz,A.N.; Sánchez,F.G.; Pareja,A.G. Resolution of deltamethrin, permethrin, and cypermethrin enantiomers by high-performance liquid chromatography with diode-laser polarimetric detection, *J.Chromatogr.Sci.*, **1998**, *36*, 210–216.

## SAMPLE

**Matrix:** fruit, vegetables
**Sample preparation:** Prepare a cleanup column by placing 4 g Florisil, 1 g activated charcoal, and a 20 mm layer of anhydrous sodium sulfate in a 400 × 10 glass column, wash with 40 mL toluene, wash with 40 mL toluene:MeCN 99:1. Homogenize 25 g chopped fruit or vegetable with 70 mL MeOH at high speed for 3 min, filter, homogenize solid with 30 mL MeOH, filter. Combine the filtrates and add them to 60 mL toluene and 300 mL 10% NaCl in water, shake well for 3 min, let layers separate. Dry the organic layer by passing it through 20 g anhydrous sodium sulfate in a 20 mm diameter column, concentrate to about 5 mL under reduced pressure at 80°, add to the cleanup column, elute with 40 mL toluene:MeCN 99:1. Evaporate the eluate just to dryness under reduced pressure at 80°, reconstitute with 1 mL MeOH, inject an aliquot. (Reflux activated charcoal (20-40 mesh) with 1 M HCl for 4 h, wash with water until the washings are neutral, dry at 95-100° (J.Assoc.Off.Anal.Chem. 1983, 66, 1013). Heat 60-100 mesh Florisil at 200° for 24 h, cool, add 4% water, mix thoroughly, store in a sealed jar (J.Assoc.Off.Anal.Chem. 1983, 66, 1003).)

## HPLC VARIABLES

**Column:** 300 × 3.9 10 μm μBondapak C18

**Mobile phase:** Gradient. MeCN:water from 62:38 to 78:22 over 32 min (Waters curve 6).
**Column temperature:** 50
**Flow rate:** 1.5
**Detector:** UV 206

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**CHROMATOGRAM**
**Retention time:** 21.05-22.08 (3 peaks)
**Limit of detection:** 50 ng/g

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**OTHER SUBSTANCES**
**Simultaneous:** allethrin, biphenthrin, fenpropathrin, fenvalerate, flucythrinate, methothrin, permethrin, Py-115, tetramethrin

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**KEY WORDS**
cucumber; tomato; cabbage; apple; pear; peach; SPE

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**REFERENCE**
Pang,G.-F.; Chao,Y.-Z.; Liu,X.-S.; Fan,C.-L. Multiresidue liquid chromatographic method for simultaneous determination of pyrethroid insecticides in fruits and vegetables, *J.AOAC Int.*, **1995**, *78*, 1474–1480.

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**SAMPLE**
**Matrix:** solutions

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**HPLC VARIABLES**
**Column:** 250 × 4.6 Pirkle type 1-A (Regis)
**Mobile phase:** Hexane:isopropanol 99.9:0.1
**Flow rate:** 1
**Detector:** UV 240 or 280

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**CHROMATOGRAM**
**Retention time:** 30-55 (various isomers)

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**KEY WORDS**
chiral

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**REFERENCE**
Cayley,G.R.; Simpson,B.W. Separation of pyrethroid enantiomers by chiral high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *356*, 123–134.

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**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Extract cotton swabs 3-4 times with 500 mL hexane, inject a 50 μL aliquot.

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**HPLC VARIABLES**
**Column:** 100 × 4 5 μm Spherisorb S5W
**Mobile phase:** Hexane:1,4-dioxane 99.8:0.2
**Flow rate:** 1
**Injection volume:** 50
**Detector:** UV 233

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**KEY WORDS**
normal phase

### REFERENCE

Eadsforth,C.V.; Bragt,P.C.; van Sittert,N.J. Human dose-excretion studies with pyrethroid insecticides cypermethrin and αcypermethrin: relevance for biological monitoring, *Xenobiotica*, **1988**, *18*, 603–614.

### SAMPLE

**Matrix:** solutions
**Sample preparation:** Inject a 10 μL aliquot of a 1 mg/mL solution in mobile phase.

### HPLC VARIABLES

**Column:** 250 × 4.6 10 μm silica (Perkin-Elmer PE-0258-0051)
**Mobile phase:** Hexane:pentane:ether 78:18.1:3.9
**Column temperature:** 25
**Flow rate:** 1.5
**Injection volume:** 10
**Detector:** UV 230

### CHROMATOGRAM

**Retention time:** 7.5, 8.5, 9.5, 11 (geometrical isomers)

### REFERENCE

Wang,Q-S.; Gao,R.-Y.; Wang,H.-Y. Computer-assisted optimization of selectivity (mobile phase, pH, and ion concentration) in high-performance liquid chromatography, *J.High Res.Chromatogr.*, **1990**, *13*, 173–177.

### SAMPLE

**Matrix:** solutions
**Sample preparation:** Inject an aliquot of a 0.1-1 mg/mL solution in hexane.

### HPLC VARIABLES

**Guard column:** 5 μm Spherisorb NH2
**Column:** 250 × 4.6 Pirkle ionic type 1-A column (Technicol)
**Mobile phase:** Hexane:isopropanol 99.85:0.15
**Flow rate:** 1.3
**Detector:** UV 230

### CHROMATOGRAM

**Retention time:** 21.4, 23.1, 25.9, 27.8, 29.0, 34.4, 35.3 (enantiomers)

### OTHER SUBSTANCES

**Also analyzed:** allethrin, fenpropathrin, fenvalerate, tetramethrin

### KEY WORDS

chiral

### REFERENCE

Lisseter,S.G.; Hambling,S.G. Chiral high-performance liquid chromatography of synthetic pyrethroid insecticides, *J.Chromatogr.*, **1991**, *539*, 207–210.

### SAMPLE

**Matrix:** solutions

### HPLC VARIABLES

**Column:** 250 × 4.6 5 μm Cyclobond I cyclodextrin-modified silica (Astec)
**Mobile phase:** MeCN:water 225:27
**Flow rate:** 1

**Detector:** UV 220

**CHROMATOGRAM**
**Retention time:** 11, 12, 13, 17, 19 (isomers)

**KEY WORDS**
comparison with GC

**REFERENCE**
Kutter,J.P.; Class,T.J. Diastereoselective and enantioselective chromatography of the pyrethroid insecticides allethrin and cypermethrin, *Chromatographia*, **1992**, *33*, 103–112.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Inject a 0.06 μL aliquot of a 1-250 μg/mL solution in mobile phase.

**HPLC VARIABLES**
**Column:** 250 × 0.5 Spherisorb ODS2 PEEK column
**Mobile phase:** Isopropanol:water 70:30 containing 10 mM ammonium acetate and 22 mM formic acid
**Flow rate:** 0.01
**Injection volume:** 0.06
**Detector:** MS, VG Quattro tandem quadrupole, electrospray, high voltage lens 550 V, sampling cone 40-120 V, collision gas argon, collision energy 25 eV, mobile phase at 5 μL/min was used as make-up flow, m/z 433

**CHROMATOGRAM**
**Retention time:** 9
**Limit of detection:** 60 pg (SIM)

**KEY WORDS**
microbore

**REFERENCE**
Fleet,I.A.; Monaghan,J.J.; Gordon,D.B.; Lord,G.A. Microbore liquid chromatography-electrospray mass spectrometry of selected synthetic pyrethroid insecticides, *Analyst*, **1996**, *121*, 55–59.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Inject 100 mL river water on to column A at 5 mL/min and let the effluent flow to waste, backflush the contents of column A on to column B and start the gradient, monitor the effluent from column B. At the end of each run backflush column A with 5 mL water, with 30 mL 100 mM pH 2 sodium citrate buffer, with 10 mL water, with 5 mL MeCN, and with 10 mL hexane:dichloromethane 50:50. Wash new column A with 10 mL water.

**HPLC VARIABLES**
**Column:** A 20 × 3 10 μm PRP-1 (Hamilton); B 30 × 4.6 10 μm RP-18 (Brownlee) + 250 × 4.6 5 μm LiChrospher C18
**Mobile phase:** Gradient. MeCN:water from 5:95 to 90:10 over 1 h, maintain at 90:10 for 4 min, return to initial conditions (?) over 1 min, re-equilibrate for 10 min.
**Flow rate:** 1 for 64 min, to 1.5 over 1 min, maintain at 1.5 for 5 min, to 1 over 5 min
**Injection volume:** 100000
**Detector:** UV 210

**CHROMATOGRAM**
**Retention time:** 59.8

**Limit of detection:** 100 ng/L

## OTHER SUBSTANCES

**Simultaneous:** alachlor, aldicarb, aldicarb oxime, atrazine, carbofuran, chlorobenzilate, chlorothalonil, chlorpyrifos methyl, chlortoluron, p,p'-DDE, DDT, deltamethrin, diazinon, diclofop methyl, dimethoate, diuron, ethofumesate, fenitrothion, fenvalerate, fluazifop butyl, fluometuron, linuron, metalaxyl, metamitron, methomyl, metobromuron, metolachlor, molinate, oxamyl, paraoxon, paraoxon methyl, parathion, parathion methyl, pendimethalin, permethrin, phenmediphan, pirimphos, pirimphos methyl, prometryne, propanil, propiconazole, simazine, terbuthylazine, trifluraline

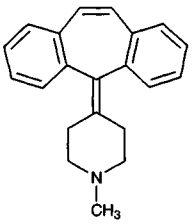
## KEY WORDS

river water; column-switching

## REFERENCE

Papadopoulou-Mourkidou,E.; Patsias,J. Development of a semi-automated high-performance liquid chromatographic-diode array detection system for screening pesticides at trace levels in aquatic systems of the Axios River basin, *J.Chromatogr.A*, **1996**, *726*, 99–113.

# Cyproheptadine



**Molecular formula:** $C_{21}H_{21}N$
**Molecular weight:** 287.40
**CAS Registry No.:** 129-03-3, 41354-29-4 (HCl sesquihydrate), 969-33-5 (HCl)
**Merck Index:** 2842
**Lednicer No.:** 1 151

## SAMPLE

**Matrix:** blood
**Sample preparation:** 100 μL Plasma + 50 μL MeOH:100 mM HCl 50:50 + 50 μL 1 μg/mL desmethyldoxepin hydrochloride in 100 mM HCl + 100 μL 1.5 M NaOH + 3 mL hexane:isoamyl alcohol 99:1, vortex for 2 min, freeze, thaw, centrifuge at 2000 g for 5 min. Remove the organic layer, repeat the extraction. Combine the organic layers and add them to 100 μL 50 mM sulfuric acid, vortex for 2 min, inject a 90 μL aliquot of the aqueous layer.

## HPLC VARIABLES

**Column:** 300 × 3.9 10 μm μBondapak C18
**Mobile phase:** MeOH:MeCN:100 mM pH 4.7 phosphate buffer containing 5 mM pentanesulfonic acid 41:15:44
**Flow rate:** 1.5
**Injection volume:** 90
**Detector:** UV 228

## CHROMATOGRAM

**Retention time:** 9.40
**Internal standard:** desmethyldoxepin (5.19)
**Limit of quantitation:** 3 ng

## OTHER SUBSTANCES

**Extracted:** metabolites

## KEY WORDS

plasma; rat

## REFERENCE

Novak,E.A.; Stanley,M.; McIntyre,I.M.; Hryhorczuk,L.M. High-performance liquid chromatographic method for quantification of cyproheptadine in serum or plasma, *J.Chromatogr.*, **1985**, *339*, 457–461.

## SAMPLE

**Matrix:** blood
**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

## HPLC VARIABLES

**Column:** 300 × 3.9 4 μm NovaPack C18
**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) $KH_2PO_4$ adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
**Column temperature:** 30

**Flow rate:** 0.8
**Injection volume:** 50
**Detector:** UV 225

## CHROMATOGRAM

**Retention time:** 7.68
**Limit of detection:** <120 ng/mL

## KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

## REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

## SAMPLE

**Matrix:** blood, tissue

**Sample preparation:** Blood or serum. 1 mL Blood or serum + 1 μg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer. Liver

homogenate. 0.5 mL Liver homogenate + 10 μg cianopramine + 500 μL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 μL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 μL aliquot of the aqueous layer.

### HPLC VARIABLES

**Guard column:** 15 × 3.2 7 μm RP-18 Newguard (Applied Biosystems)
**Column:** 100 × 4.6 5 μm Brownlee Spheri-5 RP-18
**Mobile phase:** MeCN:100 mM $NaH_2PO_4$:diethylamine 40:57.5:2.5
**Flow rate:** 2
**Injection volume:** 30
**Detector:** UV 220

### CHROMATOGRAM

**Retention time:** 30.43
**Internal standard:** cianopramine (8.93)

### OTHER SUBSTANCES

**Simultaneous:** amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranylcypromine, trazodone, trihexiphenidyl, trimipramine, triprolidine

**Noninterfering:** dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

### KEY WORDS

serum; whole blood; liver

### REFERENCE

McIntyre,I.M.; King,C.V.; Skafidis,S.; Drummer,O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, *621*, 215–223.

### SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

### HPLC VARIABLES

**Guard column:** 20 mm long Symmetry C18
**Column:** 250 × 4.6 5 μm Symmetry C8 (Waters)
**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
**Column temperature:** 30

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
**Injection volume:** 10-30
**Detector:** UV 224

**CHROMATOGRAM**
**Retention time:** 15.015

**KEY WORDS**
whole blood

**REFERENCE**
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

**SAMPLE**
**Matrix:** formulations
**Sample preparation:** Dissolve in water.

**HPLC VARIABLES**
**Column:** 250 × 4.6 Rexchrome ODS
**Mobile phase:** MeCN:MeOH:50 mM $KH_2PO_4$ 25:20:55
**Flow rate:** 2
**Injection volume:** 50
**Detector:** UV 240

**CHROMATOGRAM**
**Retention time:** 8
**Internal standard:** cyproheptadine

**OTHER SUBSTANCES**
**Simultaneous:** diltiazem

**KEY WORDS**
tablets; cyproheptadine is IS

**REFERENCE**
Shivram,K.; Shah,A.C.; Newalkar,B.L.; Kamath,B.V. Stability indicating high-performance liquid chromatographic method for the assay of diltiazem hydrochloride in tablets, *J.Liq.Chromatogr.*, **1992**, *15*, 2417–2422.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

**HPLC VARIABLES**
**Column:** 125 × 4.9 Spherisorb S5W silica
**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
**Flow rate:** 2
**Injection volume:** 20
**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

**CHROMATOGRAM**
**Retention time:** 3.9

## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

## REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

## SAMPLE

**Matrix:** tissue

**Sample preparation:** Homogenize tissue with four volumes of water. 2 mL Homogenate + 2 mL 300 mM pH 3.0 citric phosphate buffer, mix, wash with 20 mL heptane, add 2 mL 2 M NaOH, extract with 15 mL ether. Remove the organic layer and wash it with 5 mL 50 mM pH 10.0 carbonate buffer. Add the organic layer to 1 mL pH 3.0 100 mM citric phosphate buffer, mix. Remove the aqueous layer and add it to 2.5 mL 1 M NaOH, extract with 5 mL chloroform. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50 μL MeOH, inject a 10 μL aliquot. (Use chloroform with EtOH as a preservative.)

## HPLC VARIABLES

**Column:** Partisil 10/25 ODS

**Mobile phase:** MeOH:30 mM $KH_2PO_4$ 60:40
**Flow rate:** 1.4
**Injection volume:** 10
**Detector:** UV 210

**CHROMATOGRAM**
**Retention time:** 12.5
**Internal standard:** diphenylpyraline.HCl (4-diphenylmethoxy-1-methylpiperidine (9.5)
**Limit of detection:** 500 ng/g

**OTHER SUBSTANCES**
**Extracted:** metabolites

**KEY WORDS**
rat; pancreas; liver; lung; kidney; brain

**REFERENCE**
Chow,S.A.; Fischer,L.J. Metabolism and disposition of cyproheptadine and desmethylcyproheptadine in pregnant and fetal rats, *Drug Metab.Dispos.*, **1987**, *15*, 740–748.

**SAMPLE**
**Matrix:** urine
**Sample preparation:** Condition a 3 mL Suplelclean C18 SPE cartridge with 2 mL MeOH and 2 mL water. 1 mL Urine + 100-120 μL MeOH, mix thoroughly, add to the SPE cartridge, wash with two 2 mL portions of MeOH:water 80:20, elute with 6 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 1 mL MeOH, inject a 20 μL aliquot.

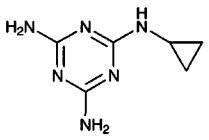
**HPLC VARIABLES**
**Column:** 150 × 6 octyl ODP
**Mobile phase:** MeOH:acetate buffer (ionic strength 0.005 I) 44:56 adjusted to pH 3.6 with acetic acid
**Column temperature:** 40
**Flow rate:** 1.4
**Injection volume:** 20
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** 8.30
**Limit of detection:** 15 ng/mL
**Limit of quantitation:** 50 ng/mL

**KEY WORDS**
SPE

**REFERENCE**
Kountourellis,J.E.; Ebete,K.O. Reversed-phase high performance liquid chromatographic determination of cyprohepadine from urine by solid-phase extraction, *J.Chromatogr.B*, **1995**, *664*, 468–471.

# Cyromazine



**Molecular formula:** $C_6H_{10}N_6$
**Molecular weight:** 166.19
**CAS Registry No.:** 66215-27-8
**Merck Index:** 2845

## SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

## HPLC VARIABLES

**Guard column:** 20 mm long Symmetry C18
**Column:** 250 × 4.6 5 μm Symmetry C8 (Waters)
**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
**Column temperature:** 30
**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
**Injection volume:** 10-30
**Detector:** UV 214.6

## CHROMATOGRAM

**Retention time:** 3.283

## KEY WORDS

whole blood

## REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

## SAMPLE

**Matrix:** solutions

## HPLC VARIABLES

**Column:** 250 × 4 10 μm Hibar RP-8
**Mobile phase:** MeCN:0.5 mM sulfuric acid 50:50
**Flow rate:** 1
**Injection volume:** 50
**Detector:** UV 214

## CHROMATOGRAM

**Retention time:** 6.89
**Limit of detection:** 20 ppb

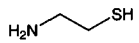
## OTHER SUBSTANCES

**Simultaneous:** melamine, metabolites

## REFERENCE

Cabras,P.; Meloni,M.; Spanedda,L. High-performance liquid chromatographic separation of cyromazine and its metabolite melamine, *J.Chromatogr.*, **1990**, *505*, 413–416.

# Cysteamine



**Molecular formula:** $C_2H_7NS$
**Molecular weight:** 77.15
**CAS Registry No.:** 60-23-1
**Merck Index:** 2848

## SAMPLE

**Matrix:** blood
**Sample preparation:** 100 μL Plasma + 500 μL 7 M pH 9.0 urea + 1 drop of 1-octanol + 50 μL 100 mg/mL sodium borohydride in 100 mM NaOH, heat at 50° for 30 min, add 500 μL cold 10% trichloroacetic acid, centrifuge for 5 min, filter (0.45 μm), pass nitrogen through filtrate for several min to exclude oxygen, inject a 20 μL aliquot.

## HPLC VARIABLES

**Column:** 250 × 4.6 5 μm BAS biophase ODS (Bioanalytical Systems)
**Mobile phase:** 50 mM Chloroacetic acid containing 3 mL/L 70% ethylamine and 60 mg/L sodium octyl sulfate, adjust the pH to 3.0 with solid monochloroacetic acid.
**Flow rate:** 1
**Injection volume:** 20
**Detector:** E, Bioanalytical Systems, gold/mercury electrode +0.15 V

## CHROMATOGRAM

**Retention time:** 8
**Limit of detection:** 50 nm

## OTHER SUBSTANCES

**Extracted:** cysteine, homocysteine, glutathione

## KEY WORDS

plasma; use stainless steel tubing between pump and reservoir; continuously purge mobile phase with nitrogen; cysteamine is the sum of cysteamine, cystamine, cysteine-cysteamine disulfide, and other cysteamine disulfides; pharmacokinetics

## REFERENCE

Smolin,L.A.; Schneider,J.A. Measurement of total plasma cysteamine using high-performance liquid chromatography with electrochemical detection, *Anal.Biochem.*, **1988**, *168*, 374–379.

## SAMPLE

**Matrix:** blood
**Sample preparation:** 150 μL Plasma + 15 μL 100 mL/L tri-n-butylphosphine in DMF, mix, let stand at 4° for 30 min, add 150 μL 100 g/L trichloroacetic acid, mix, centrifuge. Remove a 50 μL aliquot of the supernatant and add it to 10 μL 1.55 M NaOH, add 125 μL 4 mM EDTA in 125 mM pH 9.5 borate buffer, add 50 μL 1 g/L 7-fluoro-2,1,3-benzoxadiazole-4-sulfonic acid, ammonium salt (Fluka), heat at 60° for 1 h, inject a 20 μL aliquot of the supernatant.

## HPLC VARIABLES

**Column:** 4.6 mm ID 5 μm Spherisorb ODS
**Mobile phase:** MeCN:100 mM pH 2.0 $KH_2PO_4$ 4:96
**Flow rate:** 0.8
**Injection volume:** 20
**Detector:** F (wavelengths not given)

## CHROMATOGRAM

**Retention time:** 5

**Internal standard:** cysteamine

## OTHER SUBSTANCES

**Extracted:** cysteine, cysteinyl glycine, glutathione, homocysteine

## KEY WORDS

derivatization; plasma; cysteamine is IS

## REFERENCE

Kuo,K.; Still,R.; Cale,S.; McDowell,I. Standardization (external and internal) of HPLC assay for plasma homocysteine, *Clin.Chem.*, **1997**, *43*, 1653–1655.

## SAMPLE

**Matrix:** blood, tissue

**Sample preparation:** Tissue. Homogenize (Potter-Elvehjem PTFE-glass homogenizer) tissue in 20 mM EDTA, adjust to 1% (w/v) (kidney) or 2.5% (w/v) (spleen). Remove 100 μL of this solution and add it to 400 μL 100 mM pH 8.5 borate buffer, add 300 μL 0.24 mM N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide in MeCN, add 200 μL IS, heat at 60° for 30 min, cool for 5 min, centrifuge at 4° at 2000 g for 15 min, filter (Millipore 2 μm) the supernatant, inject a 20 μL aliquot of the supernatant. Plasma. Dilute rat plasma to 20% (v/v) with 20 mM EDTA. Remove 100 μL of this solution and add it to 400 μL 100 mM pH 8.5 borate buffer, add 300 μL 0.24 mM N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide in MeCN, add 200 μL IS, heat at 60° for 30 min, cool for 5 min, centrifuge at 4° at 2000 g for 15 min, filter (Millipore 2 μm) the supernatant, inject a 20 μL aliquot of the supernatant. Serum. Dilute human serum to 10% (v/v) with 20 mM EDTA. 1 mL Diluted serum + 200 μL 30% metaphosphoric acid, centrifuge at 2000 g at 4° for 20 min. Remove 500 μL of the supernatant and add it to 240 μL 2 M KOH. Remove 100 μL of this solution and add it to 400 μL 100 mM pH 8.5 borate buffer, add 300 μL 0.24 mM N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide in MeCN, add 200 μL IS, heat at 60° for 30 min, cool for 5 min, centrifuge at 4° at 2000 g for 15 min, filter (Millipore 2 μm) the supernatant, inject a 20 μL aliquot of the supernatant. (Synthesis of N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide is as follows. Add 8.8 g aluminum trichloride to 12.50 g 3-dimethylaminophenol in 185 mL chloroform and 84 g triethyl orthoformate, mix at room temperature for 10 min, when the exothermic reaction ceases add 50 mL 10% HCl, stir to hydrolyze the acetal, neutralize with 10% NaOH, filter through a short column of Celite, wash through with chloroform, wash the filtrate with saturated aqueous NaCl, dry over magnesium sulfate, concentrate under reduced pressure, recrystallize from chloroform to give 4-(dimethylamino)salicylaldehyde (mp 78-79°). Add 400 mg KOH in 3 mL EtOH to a solution of 1 g 4-(dimethylamino)salicylaldehyde and 1.3 g (?) 4-nitrobenzylbromide in 12 mL EtOH, reflux for 7 h, cool, filter to recover the crystals, wash with water, dry under vacuum, recrystallize from EtOH to give 4-dimethylamino-2-(4-nitrobenzyloxy)benzaldehyde (mp 179-180°). Add a solution of 900 mg 4-dimethylamino-2-(4-nitrobenzyloxy)benzaldehyde in 6 mL DMF to a sodium methoxide solution (prepared from 69 mg sodium in 1 mL MeOH), reflux for 20 min, add 1 mL MeOH, filter the crystals, recrystallize from EtOH to give 6-dimethylamino-2-(4-nitrophenyl)benzofuran as red needles (mp 209.5-210.5°). Reflux 1 g 6-dimethylamino-2-(4-nitrophenyl)benzofuran in 20 mL benzene (Caution! Benzene is a carcinogen!) and 18 mL MeOH containing 80 mg active carbon and a catalytic amount of ferric chloride hexahydrate for 10 min, add 2.30 g 98% hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!) dropwise, reflux for 7 h, filter, concentrate the filtrate, recrystallize from cyclohexane to give 6-dimethylamino-2-(4-aminophenyl)benzofuran as orange needles (mp 198.5-200°). Stir 605 mg 6-dimethylamino-2-(4-aminophenyl)benzofuran and 230 mg maleic anhydride in 5 mL chloroform at room temperature for 3 h, filter the crystals, wash with a small amount of chloroform, recrystallize from EtOH to obtain N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleamic acid (mp 219.5-221°). Reflux a mixture of 1.17 g N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleamic acid and 30 mg sodium acetate in 18 mL acetic anhydride, cool in an ice bath, collect the crystals of product, wash with water. Neutralize the filtrate with 20% NaOH, extract twice with 30 mL portions of chlo-

roform, wash the organic layers with saturated aqueous NaCl, dry over anhydrous magnesium sulfate, evaporate to give more product. Combine the products and recrystallize them from acetone to give N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide as reddish purple crystals (mp 203-204°) (Bull.Chem.Soc.Jpn. 1985, 58, 2192).)

**HPLC VARIABLES**
**Column:** 150 × 4.6 5 μm Toyo Soda ODS-80
**Mobile phase:** MeCN:10 mM pH 7.7 phosphate buffer 50:50 containing 30 mM tetrabutylammonium bromide
**Flow rate:** 0.8
**Injection volume:** 20
**Detector:** F ex 355 em 457

**CHROMATOGRAM**
**Retention time:** 10
**Internal standard:** disodium 6-amino-1,3-naphthalene disulfonate (3.5)
**Limit of detection:** 50 fmole

**OTHER SUBSTANCES**
**Extracted:** homocysteine, reduced glutathione (GSH), N-acetylcysteine, cysteine, coenzyme A

**KEY WORDS**
plasma; serum; rat; human; liver; kidney; spleen; derivatization

**REFERENCE**
Nakashima,K.; Umekawa,C.; Yoshida,H.; Nakatsuji,S.; Akiyama,S. High-performance liquid chromatography-fluorometry for the determination of thiols in biological samples using N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]-maleimide, *J.Chromatogr.*, **1987**, *414*, 11–17.

**SAMPLE**
**Matrix:** solutions
**Sample preparation:** 1 mL Solution + 300 μL reagent solution, let stand at room temperature for 20 min, add 500 μL 300 mM phosphoric acid solution, make up to 10 mL with water, inject a 50 μL aliquot. (Prepare the reagent solution by dissolving 3.5 mg methyl 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoate in 10 mL THF, make up to 25 mL with pH 7.5 borate buffer. Prepare methyl 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoate as follows. Dissolve 5 g 6'-methoxy-2'-acetonaphthone in warm glacial acetic acid and add 2.5 g glyoxylic acid, reflux for 24 h, evaporate to dryness under reduced pressure. Take up the residue in chloroform and extract it three times with 5% sodium carbonate solution. Combine the aqueous layers and acidify them with concentrated HCl, collect the product by filtration, recrystallize from MeOH/water or acetic acid to give 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoic acid (mp 167-9°) (Farmaco, Ed. Sci. 1982, 37, 171). Reflux 0.5 g 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoic acid, 2.5 mL MeOH, and 2-3 drops sulfuric acid in 25 mL anhydrous benzene (Caution! Benzene is a carcinogen!) for 1 h, add 20 mL water, wash the organic layer with 10 mL 5% sodium bicarbonate solution, wash the organic layer with 20 mL water. Dry the organic layer over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, purify by flash chromatography on silica gel using ethyl acetate:light petroleum (bp 40-70°) 40:60 to give methyl 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoate as a pale yellow compound (mp 116-120°).)

**HPLC VARIABLES**
**Column:** 150 × 4 5 μm Spherisorb RP-8
**Mobile phase:** MeOH:50 mM pH 3.0 triethylammonium phosphate 53:47
**Flow rate:** 1
**Injection volume:** 50
**Detector:** F ex 310 em 450

### CHROMATOGRAM
**Retention time:** 5

### OTHER SUBSTANCES
**Simultaneous:** acetylcysteine, cysteine, glutathione, homocysteine, mesna
**Noninterfering:** bacitracin, biotin, calcium pantothenate, cystine, glycine, magnesium oxide, neomycin, starch, threonine, vitamin E, pyridoxine, riboflavin phosphate

### KEY WORDS
solutions

### REFERENCE
Gatti,R.; Cavrini,V.; Roveri,P.; Pinzauti,S. High-performance liquid chromatographic determination of aliphatic thiols with aroylacrylic acids as fluorogenic precolumn derivatization reagents, *J.Chromatogr.*, **1990**, *507*, 451–458.

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### SAMPLE
**Matrix:** tissue
**Sample preparation:** 10 g Tissue + 40 mL mobile phase + 500 μL 5 mg/mL dithiothreitol in mobile phase, homogenize (VirTis Model 45 with Turbo-Shear blades (No. 16-107)) at medium setting for 2 min, centrifuge at 0-5° at 5000 rpm for 30 min, filter through glass wool, filter (Centrex 0.2 μm nylon) while centrifuging at 0-5° at 3000 rpm for 20 min, inject a 15 μL aliquot of the filtrate.

### HPLC VARIABLES
**Column:** 250 × 4.6 10 μm Partisil PXS 10/25 SCX
**Mobile phase:** MeCN:buffer 50:50 (Buffer was 200 mM formic acid, 100 mM KOH, and 0.2 mM EDTA, pH 3.5.)
**Flow rate:** 1.1
**Injection volume:** 15
**Detector:** E, Bioanalytical Systems LC-4B, Au/Hg electrode + 0.15 V, Ag/AgCl reference electrode

### CHROMATOGRAM
**Retention time:** 6.5
**Limit of detection:** 100 ppb

### KEY WORDS
pig; muscle

### REFERENCE
Pensabene,J.W.; Doerr,R.C.; Fiddler,W. Determination by liquid chromatography with electrochemical detection of cysteamine and cysteine, possible precursors of N-nitrosothiazolidine, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 1033–1035.

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### SAMPLE
**Matrix:** tissue
**Sample preparation:** Homogenize tissue in ice-cold 6% 5-sulfosalicylic acid to give a 40% tissue homogenate, centrifuge at 10000 g for 10 min. Extract the supernatant with two volumes of ice-cold chloroform:MeOH 50:50, centrifuge. Remove the aqueous layer and evaporate it to the original volume under a stream of nitrogen, adjust the pH to 5.7-6.3 with 10 M KOH, add one volume of tributylphosphine:propanol 10:90 for each 31 volumes of aqueous solution, shake gently for 1 h under a gentle nitrogen flow, adjust pH to 3 with 4% 5-sulfosalicylic acid, dilute with at least an equal volume of buffer, inject a 100 μL aliquot. (Buffer was 25 mM pH 3 monochloroacetate containing 0.5 mM sodium octyl sulfate and 0.015% EDTA.)

**HPLC VARIABLES**
**Guard column:** μBondapak C18
**Column:** 250 × 4.6 5 μm Spherisorb ODS
**Mobile phase:** MeOH:buffer 2:98 (Buffer was 25 mM pH 3 monochloroacetate containing 0.5 mM sodium octyl sulfate and 0.015% EDTA.)
**Flow rate:** 1
**Injection volume:** 100
**Detector:** E, Bioanalytical Systems LC-4B, gold/mercury amalgam electrode +0.2 V

**CHROMATOGRAM**
**Retention time:** 9
**Limit of quantitation:** 100 nM

**OTHER SUBSTANCES**
**Extracted:** cysteine, cystine

**KEY WORDS**
rat; pig; cow; liver; kidney; heart

**REFERENCE**
Garcia,R.A.G.; Hirschberger,L.L.; Stipanuk,M.H. Measurement of cyst(e)amine in physiological samples by high performance liquid chromatography, *Anal.Biochem.*, **1988**, *170*, 432–440.

**SAMPLE**
**Matrix:** urine
**Sample preparation:** Add 10 μL 50 μm 2-mercaptoethanol and 100 μL 10% trichloroacetic acid containing 10 mM EDTA to 100 μL urine, centrifuge at 760 g at 4° for 10 min. Add 350 μL 1 M pH 10.5 potassium borate buffer, 100 μL 1% tri-n-butylphosphine in water, and 100 μL 0.3% ammonium 7-fluoro-benzo-2-oxa-1,3-diazole-4-sulphonate in water to a 150 μL aliquot of the supernatant yielding a final pH of about 8.5. Incubate the mixture at 60° for 60 min, then put in an ice bath and add 50 μL 4 M HCl, inject a 10 μL aliquot of this solution.

**HPLC VARIABLES**
**Column:** 150 × 4.6 5 μm Cosmosil 5C-18AR (Nakarai Tesque, Japan)
**Mobile phase:** MeOH:75 mM pH 2.9 sodium citrate buffer 2:98
**Flow rate:** 1
**Injection volume:** 10
**Detector:** F ex 386 em 516

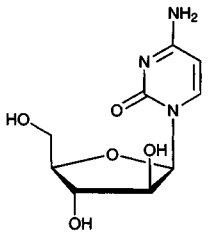
**CHROMATOGRAM**
**Retention time:** 6.0
**Internal standard:** 2-mercaptoethanol

**OTHER SUBSTANCES**
**Simultaneous:** acetylcysteine, cysteine, cysteinylglycine, γ-glutamylcysteine, glutathione, homocysteine

**KEY WORDS**
derivatization; mouse

**REFERENCE**
Oe,T.; Ohyagi,T.; Naganuma,A. Determination of γ-glutamylglutathione and other low-molecular-mass biological thiol compounds by isocratic high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.B*, **1998**, *708*, 285–289.

# Cytarabine



**Molecular formula:** $C_9H_{13}N_3O_5$
**Molecular weight:** 243.22
**CAS Registry No.:** 147-94-4, 69-74-9 (HCl)
**Merck Index:** 2853

## SAMPLE

**Matrix:** blood

**Sample preparation:** Isolate mononuclear cells from 10 mL blood by a standard step-gradient density centrifugation procedure. Wash once with PBS, resuspend in 500 μL water, add 500 μL 800 mM perchloric acid, centrifuge at 400 g for 5 min, wash the pellet with 500 μL 400 mM perchloric acid, centrifuge at 400 g for 5 min. Combine supernatants, neutralize with 10 M KOH, bring to pH 7 with 1 M KOH (Universal indicator paper), cool in ice, centrifuge at 400 g for 5 min, inject a 50-2000 μL aliquot of the supernatant. (PBS was 8.1 g NaCl, 0.22 g KCl, 1.14 g $NaHPO_4$ (sic), 0.27 g $KH_2PO_4$ in 1 L water, pH 7.4.)

## HPLC VARIABLES

**Column:** 250 × 4.6 10 μm Partisil 10 SAX

**Mobile phase:** Gradient. A was 5 mM pH 2.8 $(NH_4)H_2PO_4$. B was 750 mM pH 3.5 $(NH_4)H_2PO_4$. A:B from 70:30 to 0:100 over 30 min (concave gradient, Waters no. 9). (At the start of each day pump through 20 mL 2 M $(NH_4)H_2PO_4$ then inject 100 μL 100 mM disodium EDTA into the initial mobile phase.)

**Flow rate:** 3

**Injection volume:** 50-2000

**Detector:** UV 262

## CHROMATOGRAM

**Retention time:** 21 (as triphosphate, ara-CTP)

## OTHER SUBSTANCES

**Extracted:** F-araATP (fludarabine triphosphate), ATP, CTP, UTP, GTP

## KEY WORDS

mononuclear cells

## REFERENCE

Gandhi,V.; Danhauser,L.; Plunkett,W. Separation of 1-β-D-arabinofuranosylcytosine 5'-triphosphate and 9-β-D-arabinofuranosyl-2-fluoroadenine 5'-triphosphate in human leukemia cells by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 293–299.

## SAMPLE

**Matrix:** blood

**Sample preparation:** Add tetrahydrouridine to plasma to a final concentration of 200 μM, filter (Amicon Centricon-10 cut-off 10 000) while centrifuging at 4° at 5000 g for 30 min. Mix 50 μL ultrafiltrate with 10 μL 20 μg/mL adenosine arabinoside, inject a 50 μL aliquot.

## HPLC VARIABLES

**Column:** 250 × 4 5 μm Hypersil ODS

**Mobile phase:** MeOH:buffer 5:95 (Buffer was 50 mM pH 3.0 phosphate containing 0.4 mM sodium 1-heptanesulfonate.)

**Column temperature:** 40

**Flow rate:** 1
**Injection volume:** 50
**Detector:** UV 270

### CHROMATOGRAM
**Retention time:** 5.9
**Internal standard:** adenosine arabinoside (ara-A, vidarabine) (9.4)
**Limit of detection:** 10 ng/mL

### OTHER SUBSTANCES
**Extracted:** uracil arabinoside
**Noninterfering:** allopurinol, cephalosporins, ciprofloxacin, diazepam, metoclopramide, mitoxantrone, ondansetron

### KEY WORDS
plasma; ultrafiltrate; pharmacokinetics

### REFERENCE
Burk,M.; Volmer,M.; Fartash,K.; Schneider,W. Ion-pair liquid chromatography of cytarabine and uracil-arabinoside in human plasma, *Arzneimittelforschung*, **1995**, *45*, 616–619.

### SAMPLE
**Matrix:** blood
**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

### HPLC VARIABLES
**Column:** 300 × 3.9 4 µm NovaPack C18
**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) $KH_2PO_4$ adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
**Column temperature:** 30
**Flow rate:** 0.8
**Injection volume:** 50
**Detector:** UV 278

### CHROMATOGRAM
**Retention time:** 3.10
**Limit of detection:** <120 ng/mL

### KEY WORDS
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazo-

cine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

## REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

## SAMPLE

**Matrix:** blood, CSF

**Sample preparation:** Add tetrahydrouridine (deaminase inhibitor) to blood at a concentration of 0.1 mM, centrifuge at 800 g for 10 min, collect plasma. Add tetrahydrouridine (deaminase inhibitor) to CSF at a concentration of 0.1 mM. 0.5 mL Plasma or CSF + 0.5 μg deoxyinosine, mix, filter (Amicon MPS-1 micropartition, cut-off 10000) at 1000 g for 30 min, inject 100 μL of the ultrafiltrate.

## HPLC VARIABLES

**Guard column:** C18 (Brownlee)
**Column:** 250 × 4.6 5 μm Ultrasphere ODS
**Mobile phase:** MeOH:3,5 mM pH 3.3 $KH_2PO_4$ 2:98
**Flow rate:** 1
**Injection volume:** 100
**Detector:** UV 280

## CHROMATOGRAM

**Retention time:** 13.0
**Internal standard:** deoxyinosine (30.0) (UV 264)
**Limit of detection:** 5 (CSF), 15 (plasma)

## OTHER SUBSTANCES

**Extracted:** ara-U
**Noninterfering:** uric acid, hypoxanthine, xanthine, cytidine, deoxycytidine, uridine, deoxyuridine, MTX, folinic acid, alizapride, chlorpromazine, promazine
**Interfering:** oxypurinol, allopurinol

## KEY WORDS

plasma; ultrafiltrate

**REFERENCE**
Riccardi,A.; Servidei,T.; Lasorella,A.; Riccardi,R. High-performance liquid chromatographic assay for cytosine arabinoside and uracil arabinoside in cerebrospinal fluid and plasma, *J.Chromatogr.*, **1989**, *497*, 302–307.

**SAMPLE**
**Matrix:** bulk, formulations
**Sample preparation:** Bulk. 3 mg Bulk drug + 5 mL 1.4 mg/mL p-toluic acid in MeOH + 25 mL mobile phase, inject a 10 μL aliquot. Formulations. Make up vial with sterile water to give a 1 mg/mL solution. Add 3 mL of this solution to 5 mL 1.4 mg/mL p-toluic acid in MeOH and 20 mL mobile phase, inject a 10 μL aliquot.

**HPLC VARIABLES**
**Column:** 300 × 3.9 μBondapak C18
**Mobile phase:** MeOH:water 5:95 containing 1.34 g/L $Na_2HPO_4$ and 0.71 g/L $NaH_2PO_4.H_2O$, apparent pH 7.0
**Flow rate:** 1
**Injection volume:** 10
**Detector:** UV 254

**CHROMATOGRAM**
**Retention time:** 7
**Internal standard:** p-toluic acid

**OTHER SUBSTANCES**
**Simultaneous:** cyclocytidine, azacitidine, uracil arabinoside, cytosine, azacytosine, metabolites

**REFERENCE**
Kissinger,L.D.; Stemm,N.L. Determination of the antileukemia agents cytarabine and azacitidine and their respective degradation products by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *353*, 309–318.

**SAMPLE**
**Matrix:** cell suspensions
**Sample preparation:** Centrifuge cell suspension at 400 g, suspend the pellet in 0.9% NaCl, centrifuge, discard the supernatant. Add 120 μL MeCN:2.5 mM PIC A low UV (Waters) 0.2:99.8 (pH 3.0) to the pellet, vortex vigorously, centrifuge, filter (5 μm)

**HPLC VARIABLES**
**Guard column:** 30 × 5 5 μm C18 (Macherey-Nagel)
**Column:** 250 × 4.6 5 μm C18 (Macherey-Nagel)
**Mobile phase:** Gradient. A was MeCN:2.5 mM PIC A low UV (Waters) 0.2:99.8, pH 3.0. B was MeCN:100 mM $KH_2PO_4$ containing 5 mM PIC A low UV (Waters) 0.5:99.5, pH 2.7. A:B 100:0 for 17 min, to 0:100 (step gradient), after 28 min re-equilibrate at 100:0 at 1.5 mL/min for 10 min.
**Column temperature:** 21
**Flow rate:** 0.8 for 18 min, to 1.5 over 1 min, maintain at 1.5 for 26 min
**Injection volume:** 500
**Detector:** radioactivity (calcium fluoride solid scintillator)

**CHROMATOGRAM**
**Retention time:** 5.24
**Limit of detection:** 40 pg

**OTHER SUBSTANCES**
**Extracted:** metabolites

**KEY WORDS**
tritium labeled

**REFERENCE**
Braess,J.; Pfortner,J.; Kaufmann,C.C.; Ramsauer,B.; Unterhalt,M.; Hiddemann,W.; Schleyer,E. Detection and determination of the major metabolites of [3H]cytosine arabinoside by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *676*, 131–140.

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**SAMPLE**
**Matrix:** formulations
**Sample preparation:** Dilute with mobile phase, inject an aliquot.

**HPLC VARIABLES**
**Column:** 250 × 4.6 5 μm C18
**Mobile phase:** MeCN:100 mM $NaH_2PO_4$ 20:80 adjusted to pH 4.2 with phosphoric acid
**Flow rate:** 2.5
**Injection volume:** 20
**Detector:** UV 300

**CHROMATOGRAM**
**Retention time:** 1.45

**OTHER SUBSTANCES**
**Simultaneous:** fluorouracil, granisetron

**KEY WORDS**
stability-indicating; injections; saline

**REFERENCE**
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

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**SAMPLE**
**Matrix:** solutions
**Sample preparation:** Inject a 20 μL aliquot

**HPLC VARIABLES**
**Column:** 250 × 5 5 μm Spherisorb 5 ODS
**Mobile phase:** 10 mM $KH_2PO_4$ adjusted to pH 7
**Flow rate:** 1.3
**Injection volume:** 20
**Detector:** UV 275

**CHROMATOGRAM**
**Retention time:** 9

**OTHER SUBSTANCES**
**Simultaneous:** cytidine, cytosine, aza-analogs

**REFERENCE**
Romanová,D.; Novotny,L. Chromatographic properties of cytosine, cytidine and their synthetic analogues, *J.Chromatogr.B*, **1996**, *675*, 9–15.